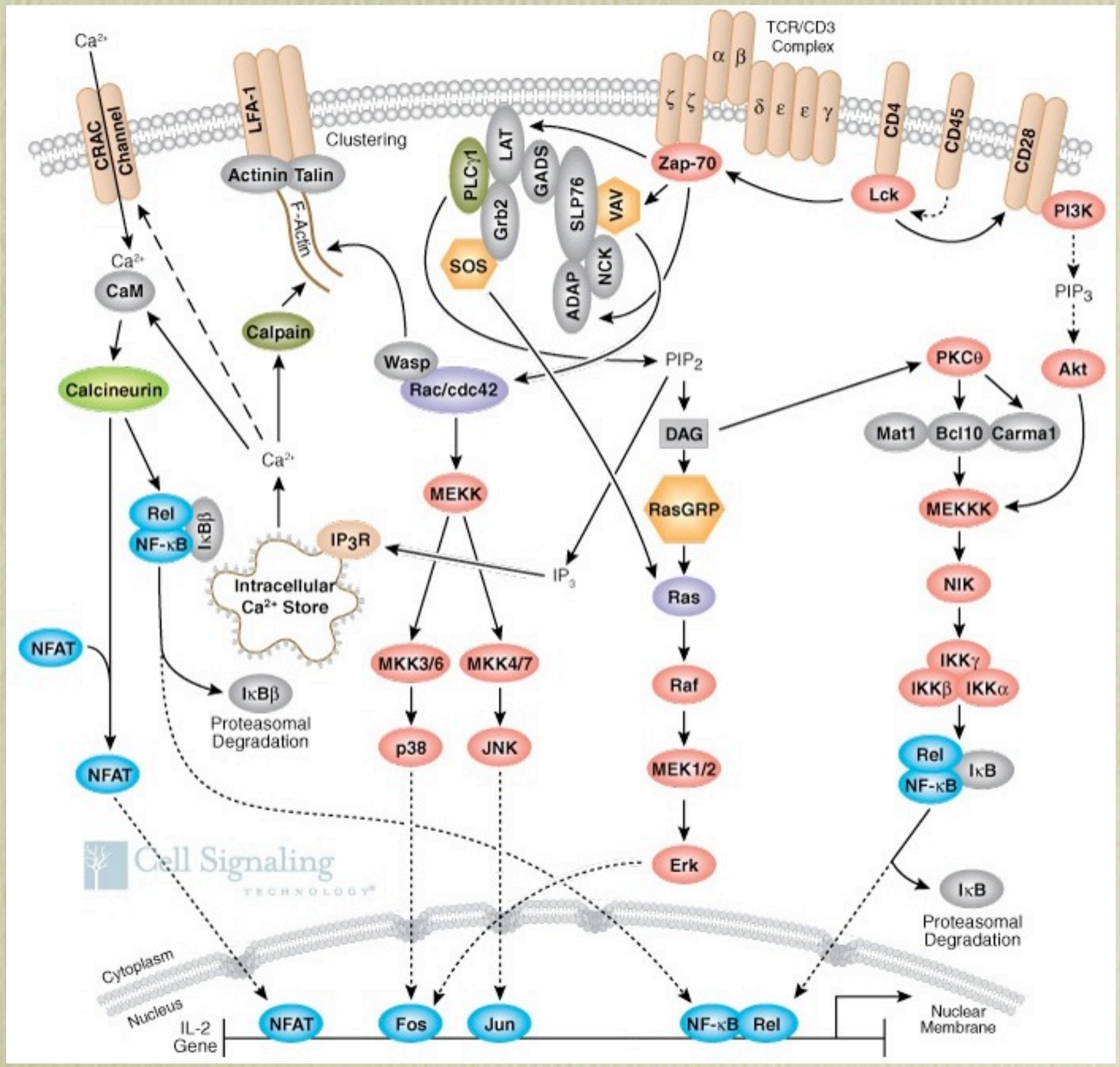
Quantifying membrane-receptor binding kinetics using single particle tracking data

Raibatak Das

Jennifer Morrison, Christopher Cairo and Dan Coombs

Membrane receptor dynamics



[www.cellsignaling.com]

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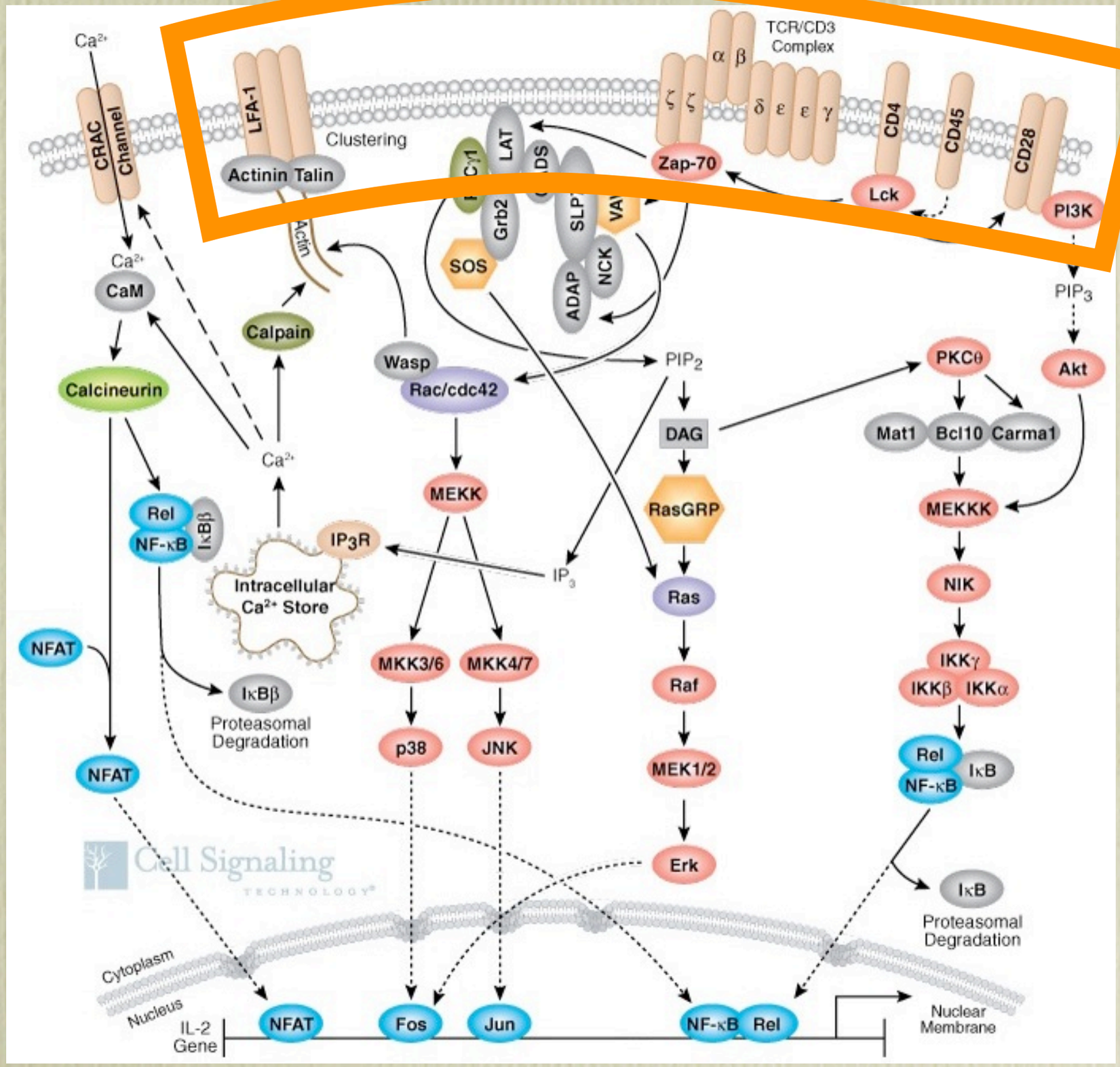

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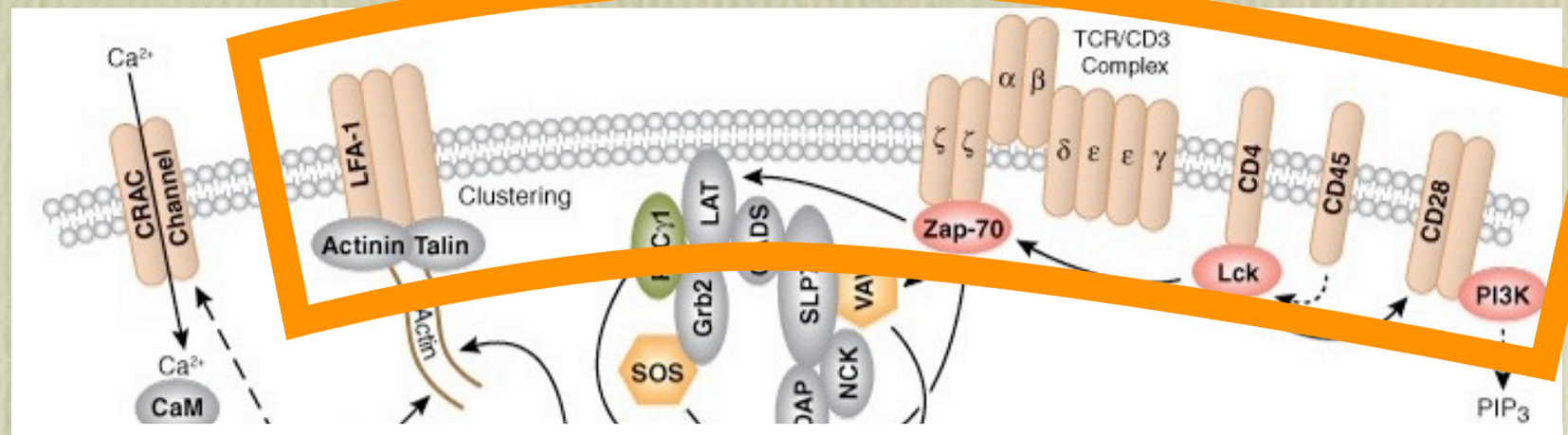
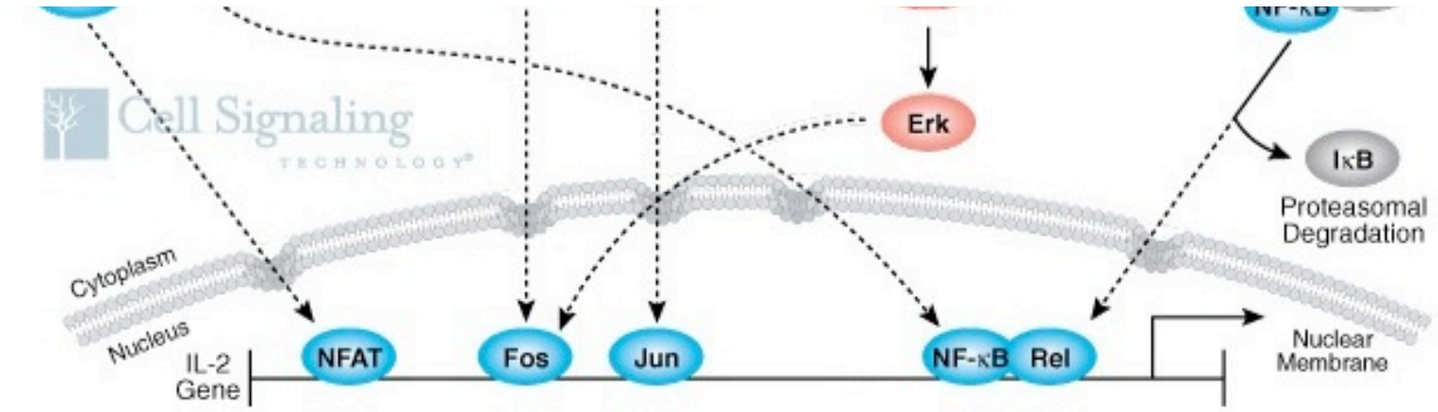


What information does membrane-receptor dynamics (specifically, receptor mobility) reveal about signaling transduction



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What factors affect receptor mobility?

- Molecular characteristics (size, number of transmembrane domains).
- Receptor aggregation.
- Membrane organization and heterogeneity.
- **Interactions with other proteins, eg: cytosolic signaling proteins.**

Single particle tracking

Technique to observe dynamics of optically tagged biomolecules with high spatiotemporal resolution.

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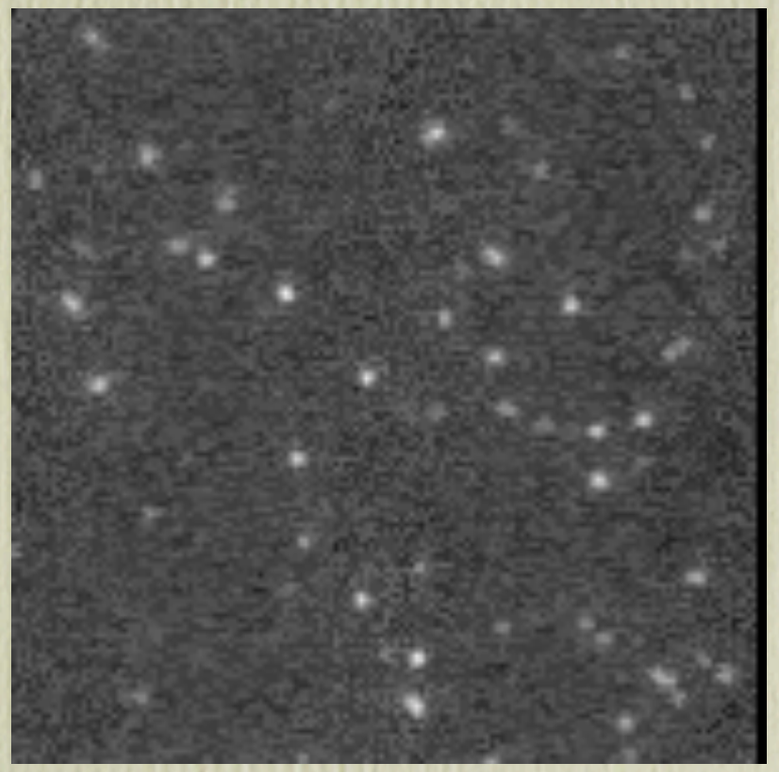

murine polyoma virus–like particles on live mouse fibroblasts.

[Ewers et. al. *PNAS*, **102**, 15110-15]

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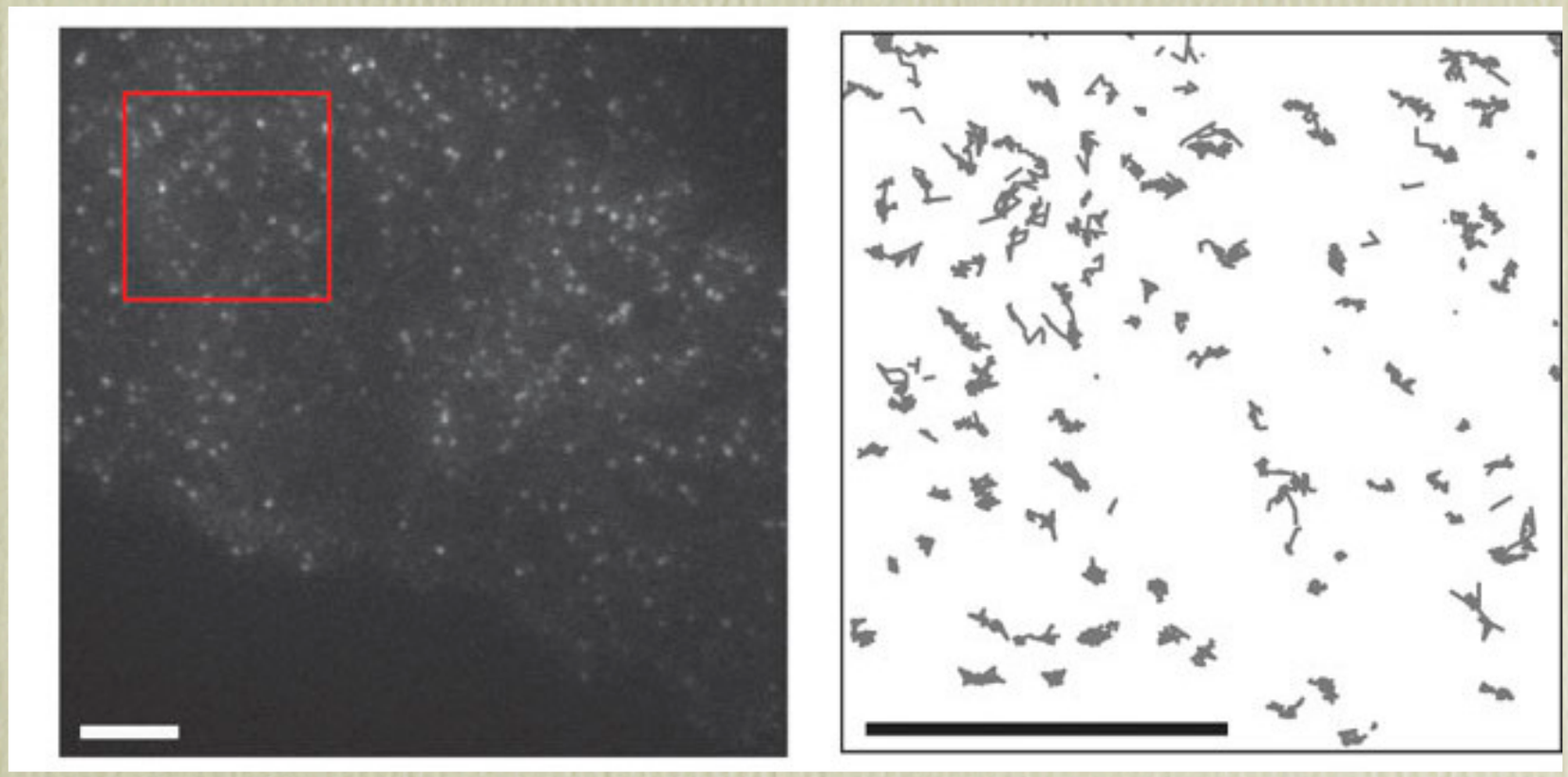

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LFA-1

- Cell surface integrin receptor.
- Binds to ICAM-1.
- Mediates immune synapse formation between a T cell and an APC
- Interacts with the actin cytoskeleton to modulate lymphocyte adhesion and migration.

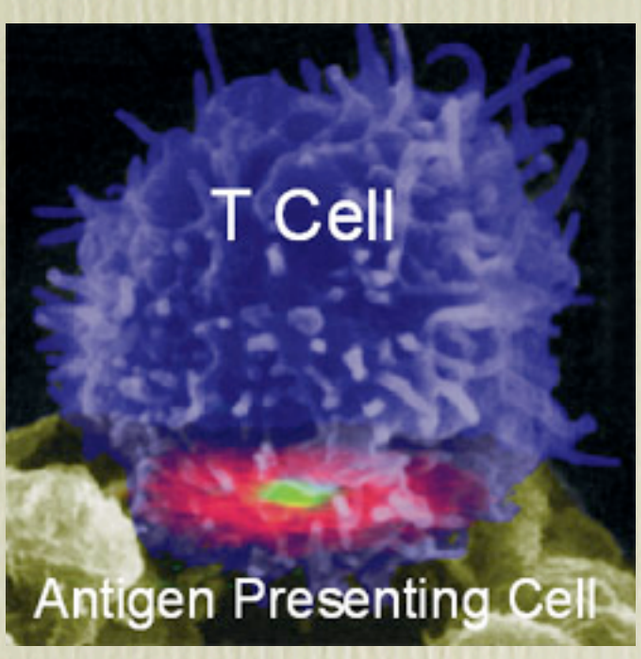
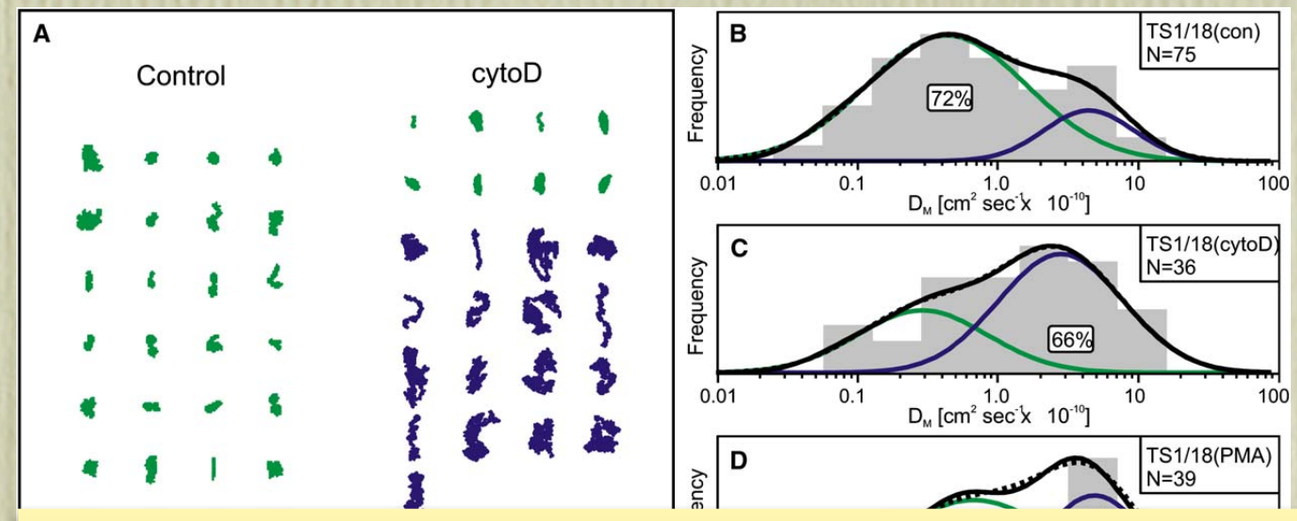


Image from Dustin lab

SPT analysis of LFA-1

Previous analysis of LFA-1 trajectories on Jurkat cells revealed two diffusive states.



- Trajectories classified based on the fits of the observed mean squared displacement to the following equation:

$$MSD(t) = 4Dt^{\alpha}$$

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Data were acquired at 1000 Hz.

This and other evidence points to interactions between LFA-1 and the actin cytoskeleton that modulates LFA-1 mobility.

There may be transient changes within each trajectory, on a shorter timescale relative to the total acquisition time.

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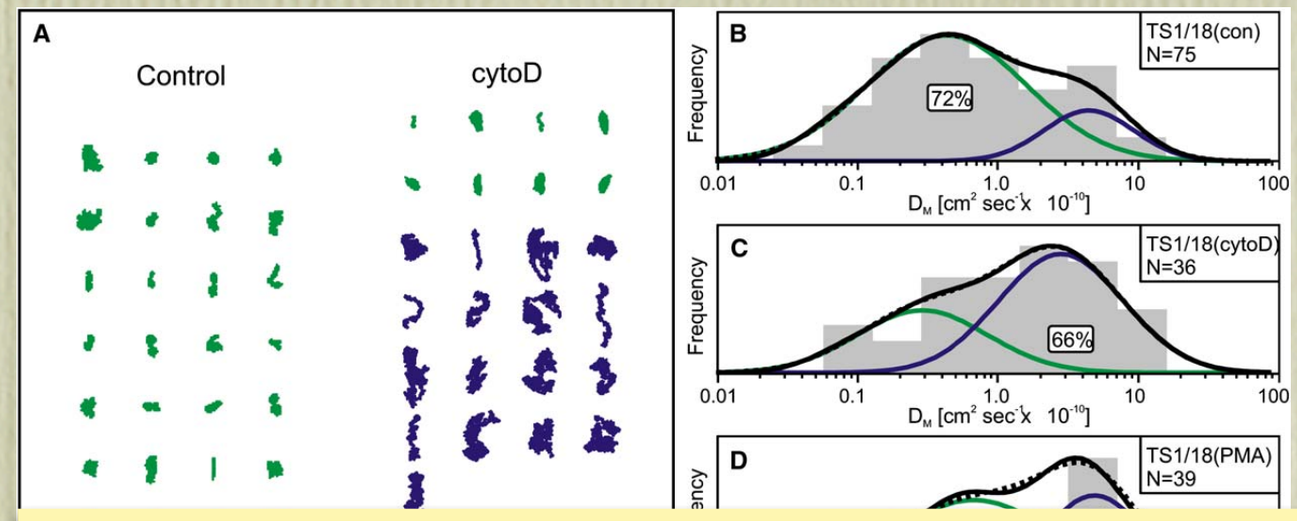

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There may be transient changes within each trajectory, on a shorter timescale relative to the total acquisition time.

This analysis captures an equilibrium distribution, but not the dynamic transitions between the two states.

A 2-state diffusion model

We developed a 2-state model to capture the dynamics of LFA-1 interactions with the actin cytoskeleton.

$$P + S \underset{k_{\text{off}}}{\overset{k_{\text{on.true}}}{\rightleftharpoons}} C$$

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Model assumptions:

1. Spatially homogeneous substrate distribution.

2. Distinct diffusion coefficients for the two states.

3. Particle state changes nearly instantaneously (relative to the acquisition frame rate), and only at the acquisition time.

Consequences of the assumptions:

Spatially homogeneous substrate distribution.

Effectively first order binding with a forward rate constant

$$k_{\text{on}} = k_{\text{on.true}}[S]_{\text{eq}}$$

Distinct diffusion coefficients for the two states.

$$D_1 \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftarrows}} D_2$$

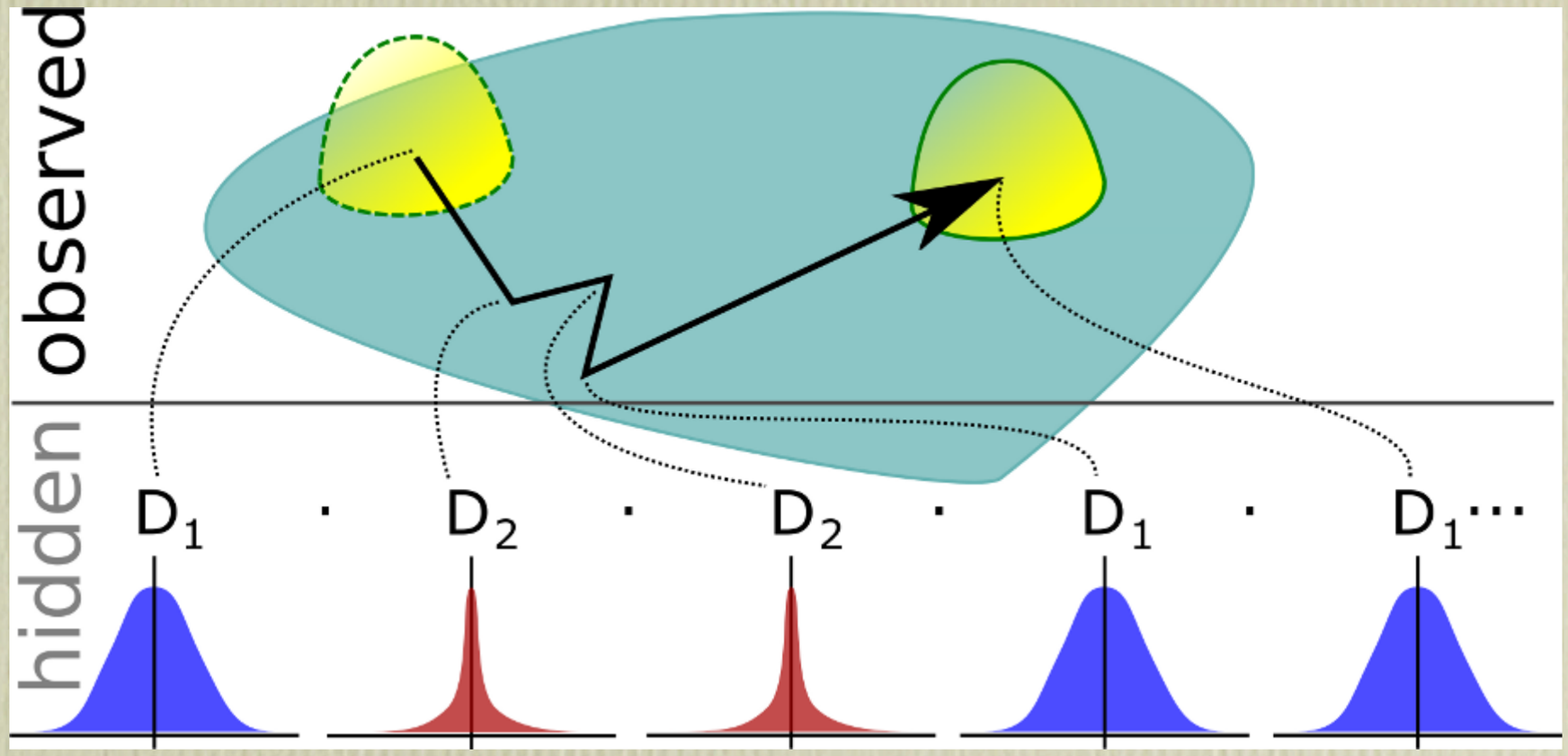
Particle state changes nearly instantaneously (relative to the acquisition frame rate), and only at the acquisition time.

$$p_{12} = \frac{k_{\text{on}}}{k_{\text{on}} + k_{\text{off}}}\left[1 - e^{-(k_{\text{on}}+k_{\text{off}})\tau}\right]$$

$$p_{21} = \frac{k_{\text{off}}}{k_{\text{on}} + k_{\text{off}}}\left[1 - e^{-(k_{\text{on}}+k_{\text{off}})\tau}\right]$$

Mention that tau = 1/frame

A 2-state hidden Markov model



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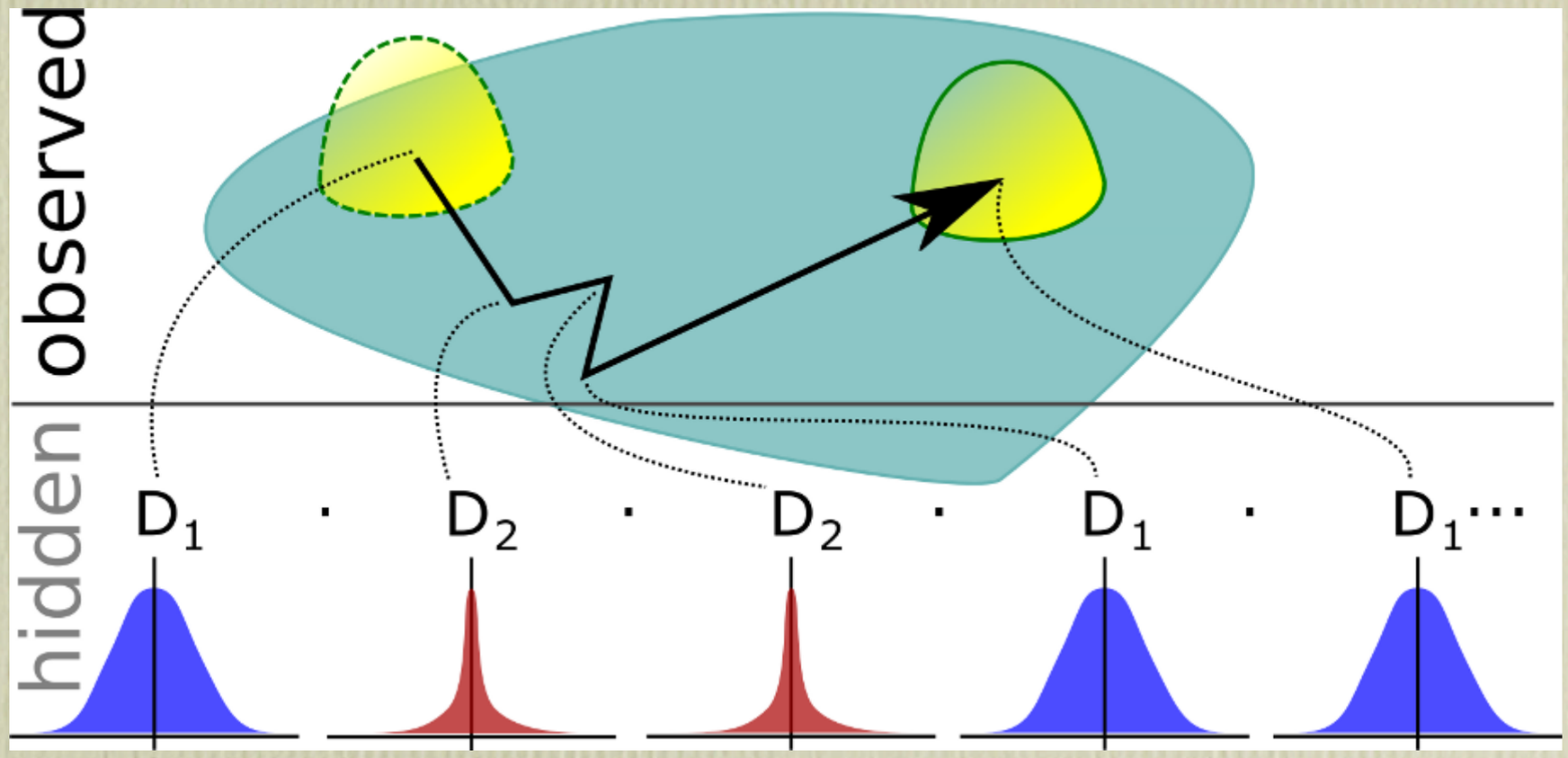
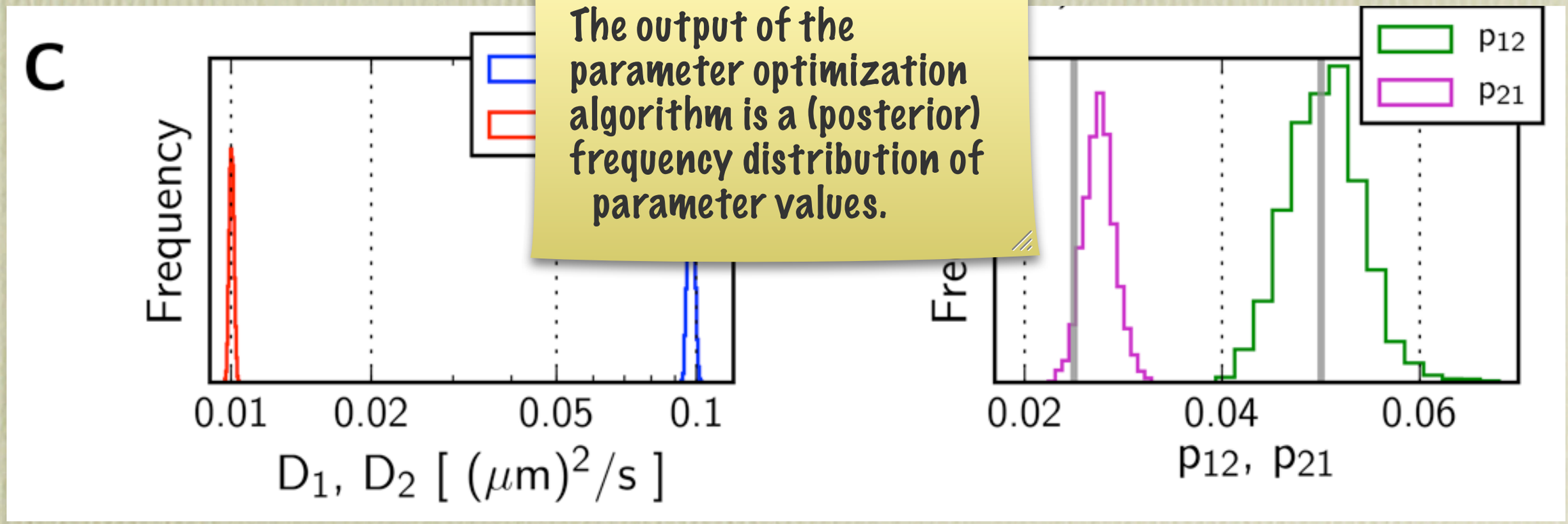


- Model parametrized by $\theta = \{D_1, D_2, p_{12}, p_{21}\}$
- The likelihood function $L[\theta|O]$ describes how likely it is to observe the given set of tracks.
- An efficient algorithm exists to compute the likelihood function for a given set of parameter values.

HMM parameter optimization

We use a stochastic likelihood maximization scheme to determine the most likely parameter values for an observed set of tracks.



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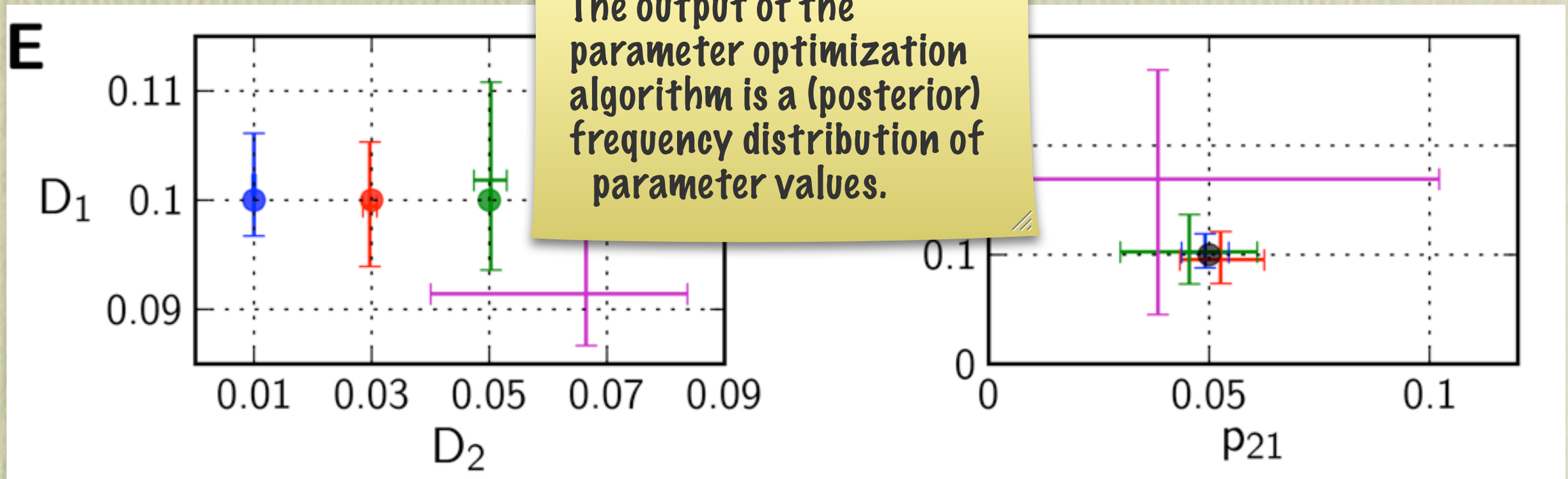

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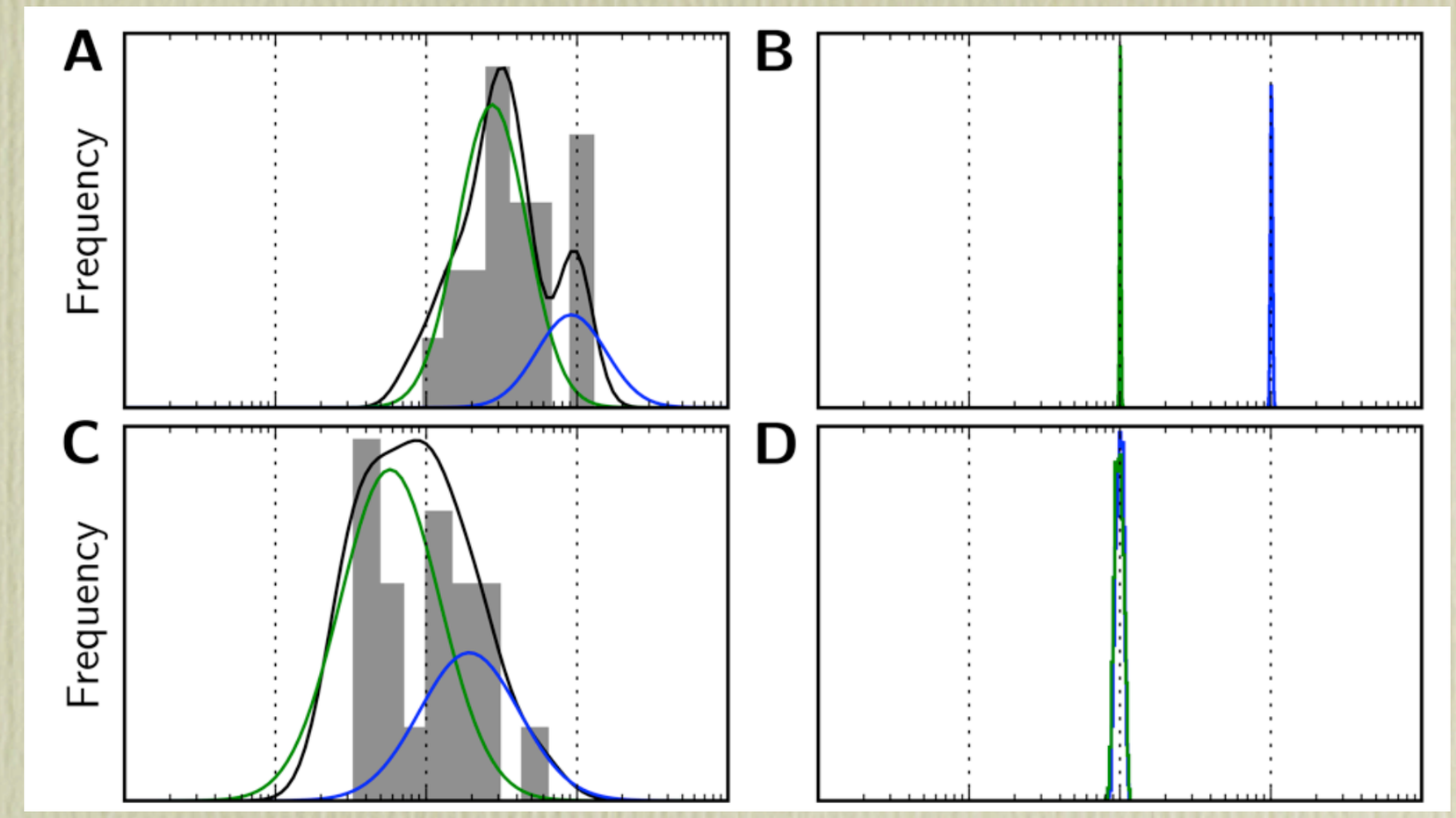
Extensive tests with simulated particle tracks show that this analysis is robust over a wide range of parameters, provided $D_1 > 2D_2$

HMM vs. MSD analysis



- Greater precision in estimating diffusion coefficients.
- Transition probabilities contain kinetic information (k_{on} and k_{off})
- Possible to statistically test for the most optimal model using AIC.

Application to LFA-1 data

2-state model is substantially statistically preferred over a 1-state model for LFA-1 mobility.

Justifications for HMM

D1 and D2 are well separated.

p1 2 and p21 << frame rate

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What biological insights does the HMM analysis provide?

Treatment	D_1	D_2	p_{12}	p_{21}	$K=k_{on}/k_{off}$	D_{eff}
Control	0.081	0.015	3.9	9.8	0.4	0.062
Cytochalasin D	0.088	0.019	2.5	11	0.22	0.076
PMA	0.057	0.008	19	23	0.81	0.035

ICAM-1 ligated LFA-1 on Jurkat T cells.

* D_{eff} is the diffusion constant for a 1-state model.

Units: Diffusion coefficients in $\mu m^2/s$.
Transition probabilities in *Hz*.

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- Disrupt … ton shifts the equilibr …

- Multipl … ed activation:
  1. Possible changes in binding partner(s).
  2. Dynamic remodelling of the actin cytoskeleton.

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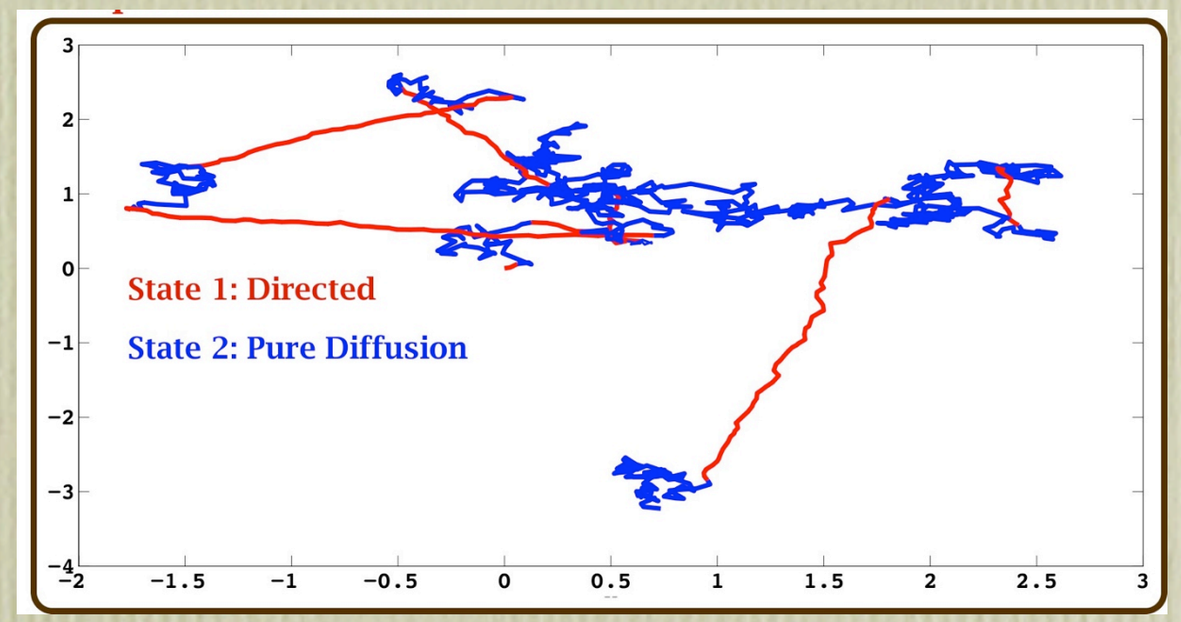
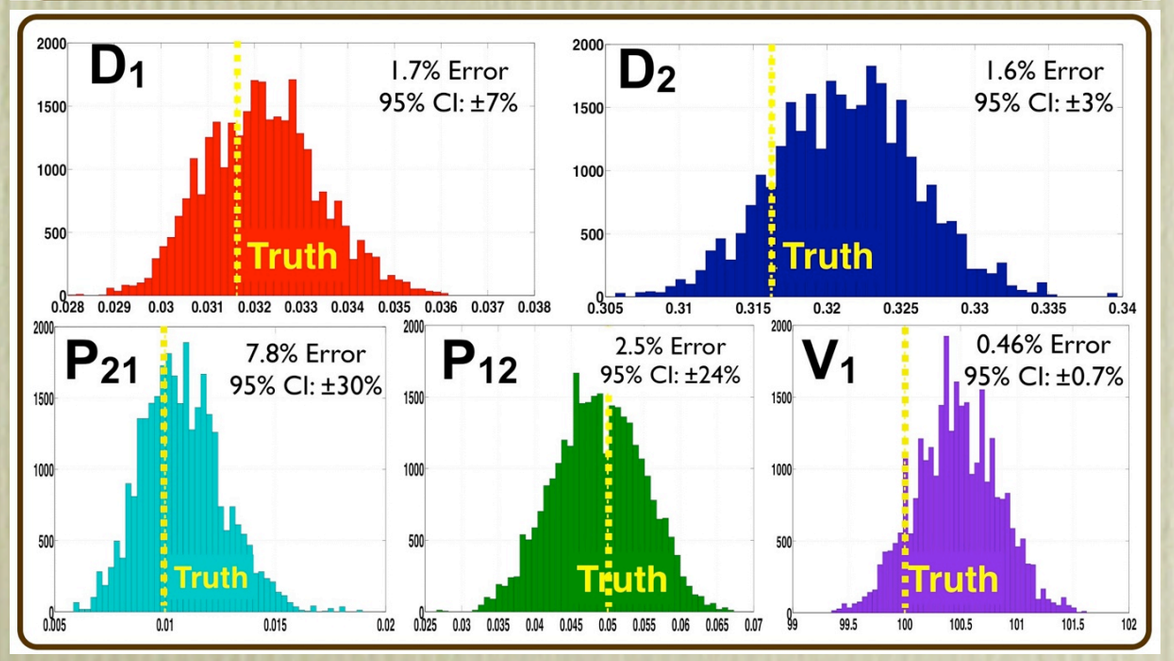
Further developments

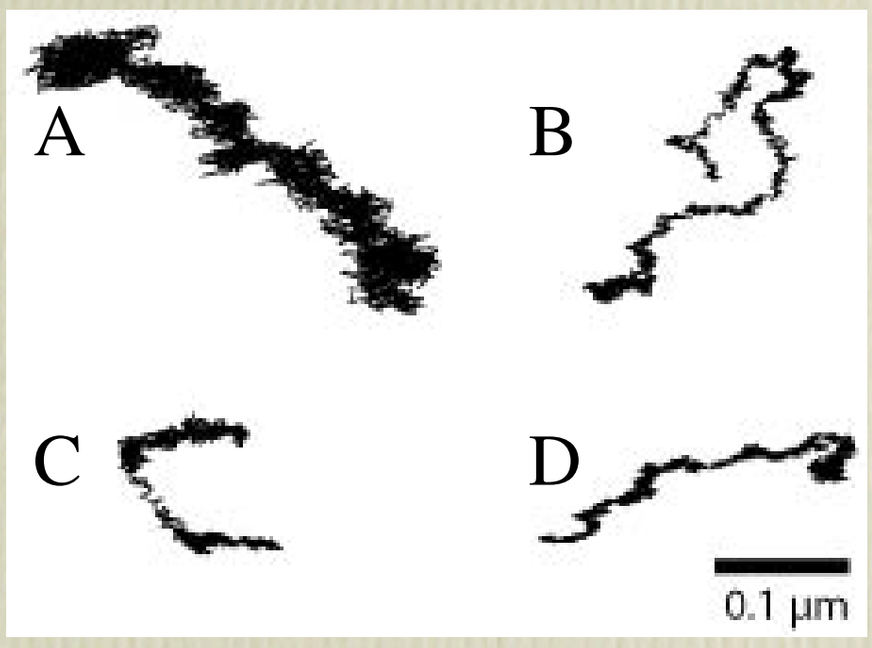
The basic 2-state diffusion model can be extended to include additional states and other modes of motion, eg: drift + diffusion.



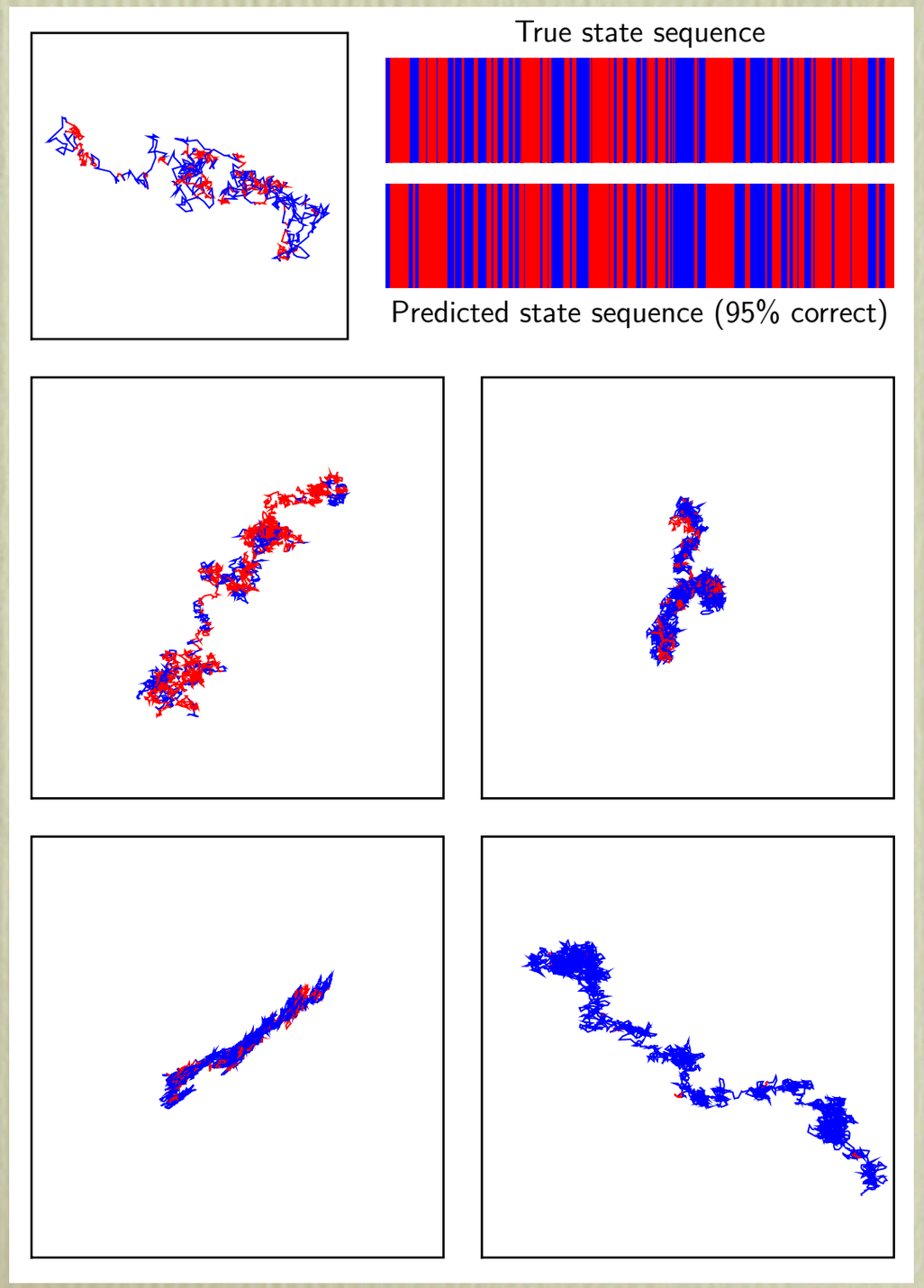


Analysis of LFA-1 and CD45 trajectories does not support a model with directed motion

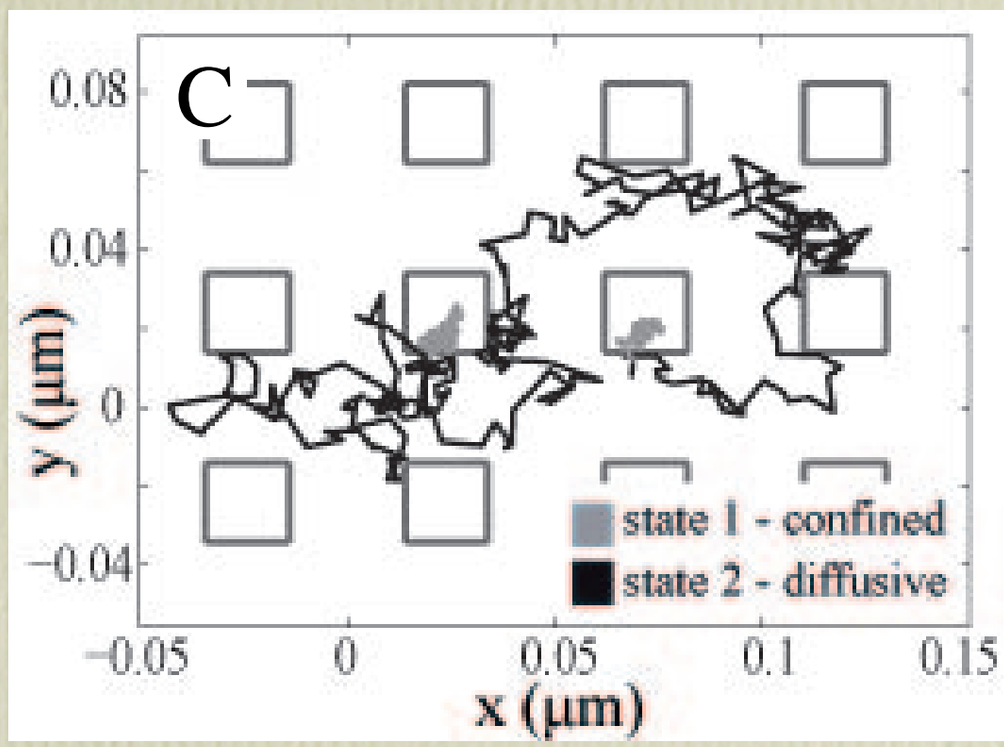
[Analysis and figures by Jennifer Morrison]



Toward a spatial model: Trajectory segmentation



- The 2-state HMM analysis allows us to infer the most probable sequence of state switching within individual trajectories
- Experimental LFA-1 trajectories show a range of switching behaviour, suggesting a role for spatial heterogeneity and/or confinement.



Summary

The hidden Markov analysis exposes transient changes in mobility that occur on a short time scale within single trajectories.

The likelihood-based model comparison allows for optimal model selection.

LFA-1 dynamics are well-described by a 2-state model with a reduced mobility state due to LFA-1 interactions with the actin cytoskeleton that undergo large-scale changes upon cellular activation.

The HMM analysis can be extended to test for transient drift and spatial confinement.

[Das, Cairo and Coombs, *PLoS Comp Biol*, **5**,e1000556]

[Morrison, Das, Cairo and Coombs (in preparation)]

Acknowledgements

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- Jennifer Morrison
- Christopher Cairo
- Golan Lab
- Gerda deVries, Vishaal Rajani, Gustavo Carrero